

**Mechanisms behind interplant communication -
'how do plants talk?'**

TOWARDS AN INTEGRATION OF THE MECHANISMS OF INTERPLANT
COMMUNICATION

MASTER THESIS

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Abstract

When plants are under herbivore or pathogen attack, or experiencing abiotic stress conditions, they can emit signalling molecules that can be received and perceived by their neighbouring plants. Receiving plants can respond to such signals by inducing defence responses, changing growth or priming their defences for possible future attack. Such transfer of information between plants, also named 'interplant communication', can take place above or below ground. Above ground signalling molecules are transported through air as blends of volatile compounds of specific concentrations. Below ground, plants can send and perceive signals through roots or common mycorrhizal networks.

Numerous studies performed over the last three decades brought better understanding of the identity of signaling molecules and mechanisms behind the emission and perception of them. However there is still a lack of information, especially on the molecular mechanisms of signalling pathways involved in the signal emission, perception and processing. Furthermore, most of the published studies have so far focused on the specific types of signalling taking place either above or below ground, considering plant tissues as independent systems. This thesis presents review of all currently known mechanisms of interplant communication, the circumstances under which these mechanisms are triggered and the signal molecules that are involved. Different mechanisms of interplant communication are discussed in integrated manner, taking into account plants as whole organisms that are able to perceive and simultaneously respond to several stimuli.

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Introduction

The ability of plants to emit and intercept signalling molecules from other plants has been one of the developing research topics in molecular and plant ecology over the last three decades. Ever since Baldwin and Schultz¹ as well as Rhoades² provided first indications of interplant communication, questions about community evolution³, circumstances under which plants exhibit such behaviour and questions on physiological and molecular mechanisms behind it arose. With more and more evidence that plants are able to perceive and respond to the signals released from neighbouring plants also in the natural environment⁴⁻⁸, interplant communication is becoming a widely recognised and accepted phenomena. Many of its aspects however remain somewhat controversial. It is still debated whether plants deliberately send messages to their neighbours, or whether interplant communication is a side effect of other functions of signalling molecules. Furthermore, it is unknown what this means from an evolutionary perspective⁹. Current general opinions lean toward the idea of plants 'eavesdropping' on volatile organic compounds (VOCs) of their neighbours and consequently responding by adapting their phenotypes and therefore enhancing their fitness. Supporting such a concept further is also recently elucidated within-plant signalling through aerial space, mediated by similar compounds as found to induce response in neighbouring plants¹⁰.

At the same time there are also an increasing number of reports on the different mechanisms of interplant communication. Besides well established airborne communication other mechanisms of interplant communication have been observed below ground. Modern approaches and techniques are enabling better understanding of the identity of signalling molecules, as well as molecular mechanisms leading to their release, their perception and formation of the response by the receiving plant. Wide transcriptional analyses can be used to connect biochemical, ecological and physiological data within a genetic framework.

Most of the published data on interplant communication has so far focused on specific types of signalling taking place either above or below ground. However, since plant tissues do not act as independent organisms, an integrated approach is needed to fully understand how plants signal to one

another. In this thesis I first review all currently known mechanisms of interplant communication, the circumstances under which these mechanisms are triggered and the signal molecules that are involved. This is essential to establish where the data is lacking, which questions still need to be answered and how to proceed. Secondly, I compare and discuss currently known data in the integrated manner, placing different types of interplant communication in the same context and trying to discuss their impact on each other, taking into account plants as whole organisms that are able to perceive and simultaneously respond to several stimuli.

Volatile signalling as mechanism of interplant communication

With more than 1700 currently known compounds¹¹, volatiles play an important role in the mechanisms of plant interaction within the environment. They are part of plant defence and reproductive mechanisms, involving interactions of plants with organisms of higher trophic levels (**Fig. 1**). The first implications of interplant communication, in which herbivore resistance was shown to increase in plants growing in close range to herbivore-attacked plants^{1,2}, was suggested to be mediated through air-borne signals¹². These pioneer findings caused mixed responses in public and the topic was popularly named 'talking trees'.

Since then, new evidence has been gathered under laboratory, as well as field conditions. This evidence has confirmed that plants respond to volatile airborne molecules emitted from other plants and identified to some extent identity of molecules and mechanisms of action involved. The emission and perception of VOCs are currently one of the most researched and acknowledged types of interplant communication.

STARTING THE CONVERSATION

Cues for interplant signalling, their recognition and signalling pathways leading to VOCs synthesis

What are the cues causing plants to release VOCs which are able to affect their neighbouring plants and what is the mechanism behind their perception and recognition? As sessile organisms, plants had to develop diverse and numerous defensive

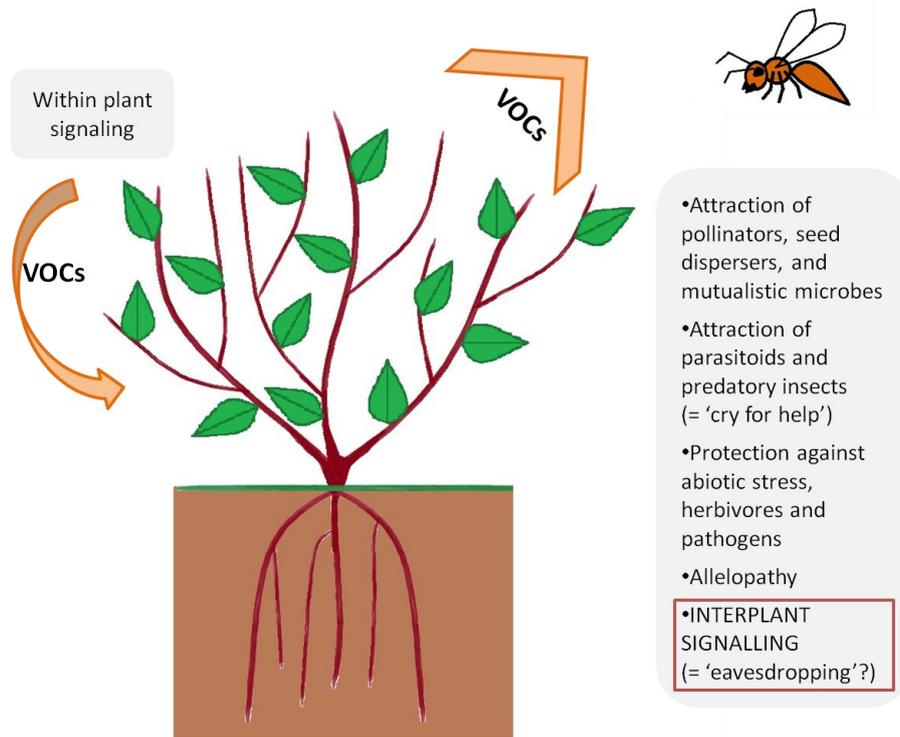


Fig. 1: Roles of the plant volatile organic compounds (VOCs) in interaction of plants within the environment. VOCs play part in defence responses, such as a 'cry for help' and protection under abiotic or biotic stress conditions, transfer of information within and between plants and in reproductive mechanisms, such as attraction of pollinators and seed dispersal.

mechanisms in order to survive constant attacks by herbivores and pathogens. Therefore it is not very surprising that most plants shown to be emitters of airborne VOCs involved in the interplant communication are responding to damage caused by attackers.

RESPONSE TO HERBIVORY

Herbivore feeding has been known to invoke defence responses in the neighbours of attacked plants since the first observations of interplant communication. In 1983 David F. Rhoades, one of the pioneers in the field of plant-plant signaling, reported that Sitka willow (*Salix sitchensis*) trees respond to their neighbouring willow trees, attacked by tent caterpillars (*Malacosoma californicum-pluviale*). Because no root connections between trees were found, Rhoades assumed that neighbouring trees are able to sense airborne signals emitted from the damaged willows². Subsequently, numerous researchers have shown that plants can intercept and respond to herbivore-induced plant volatiles (HIPVs) in response to attacks by herbivorous arthropods.

Several experiments, where lima bean plants (*Phaseolus lunatus*) were infested with spider mites (*Tetranychus urticae*, Acari), showed that receiving lima beans responded to volatile compounds from attacked plants¹³⁻¹⁵. HIPVs released from lima bean plants that were infested with the leaf miners caused a defence response in exposed *Arabidopsis thaliana* plants¹⁶. Damage caused by several species of beetles (Coleoptera) was also shown to be able to induce release of HIPVs that resulted in defence responses in receiving plants; cereal leaf beetle (*Oulema melanopus*) in the common wheat (*Triticum aestivum*), common oat (*Avena sativa*) in barley plants (*Hordeum vulgare*)¹⁷ and *Gynandrobrotica guerreroensis* and *Ceratomyxa ruficornis* beetles in the lima bean plants¹⁰. Other herbivorous arthropods, reported to be involved in interplant communication, include aphids (*Nasonovia ribis-nigri*)^{18,19}, whiteflies (*Trialeurodes vaporariorum*)²⁰ and armyworms (*Spodoptera exigua*, *Mythimna pseudalexia*)^{14,21}.

Tissue damage is the most obvious consequence of an herbivore attack. It has been shown that mechanical wounding, clipping or defoliation of some plant species can be sufficient to induce a

release of the volatile compounds responsible for transfer of the information between plants^{8,22–30}. An experiment performed by Arimura and his colleagues however showed a full response of receiving lima bean plants only when they were exposed to volatiles from the spider mite infested conspecific leaves and not when emitting leaves were artificially wounded¹³. The finding that mechanical wounding of plants can be insufficient for a complete induction of HIPVs was confirmed also by Piesik, who compared VOC responses of several cereal plants that were either infested by the cereal leaf beetles or mechanically injured in form of the scrapping injury, simulating the pattern of beetle tissue damage. Plants exposed to herbivore not only released higher concentrations of volatiles, but also emitted 13 additional specific HIPVs¹⁷. On the other hand, it has been showed that continuous mechanical damage, induced by a robotic device (MecWorm), resulted in release of HIPVs that perfectly matched HIPVs released as response to caterpillar (*Spodopteralittoralis*) feeding³¹.

What can we therefore conclude about the mechanism behind induction of the plant signalling HIPVs? Mentioned examples fit into the current knowledge on general plant-herbivore interactions, perception of herbivores and signalling mechanisms that result in the 'induced resistance', induction of broad spectrum of plant defence systems, including release of HIPVs.

Mechanical wounding of plants results in a disruption of plant tissue and damaging of plant cells, causing molecules from different compartments to come in the contact with each other. According to Heil this can trigger a 'plant damaged-self recognition', resulting in induction of jasmonic acid (JA) signalling pathways³². Endogenous plant signals (also named damage-associated molecular patterns, DAMPs) can therefore act as elicitors to induce plant immune response³³. Upon herbivore feeding, plants perceive and respond to herbivory associated molecular patterns (HAMPs) i.e. herbivore-derived elicitors from oral secretions of feeding herbivores and possibly HAMPs originated from a specific pattern of wounding^{reviewed in 34}. Several fatty acid-amino acid conjugates (FACs) derived from herbivore saliva, such as volicitin (conjugate of 17-hydroxylinolenic acid and L-glutamine)^{35,36}, and also other chemical compounds such ascaeliferins³⁵, disulfide-bridged peptides (inceptin) and β -glucosidase^{reviewed in 37}, have been shown to act as elicitors derived from chewing

herbivores (caterpillars etc.). The mode of action^{reviewed in 38–40} of such molecules is usually connected with the influx of Ca^{2+} in plant cells and therefore the depolarisation of their plasma membrane, which can be caused by action of elicitor receptors⁴¹ or, in case of some FACs, due to their amphiphilic nature, resulting in the formation of channel-like structures in the membrane. Ca^{2+} ions can further take part in signalling through Ca^{2+} sensing proteins (e.g. calmodulins and calmodulin-like proteins) and MAPK cascades (e.g. wound-induced protein kinase, WIPK, and salicylic acid-induced protein kinase, SIPK), resulting in the induction of primarily JA and ethylene signalling pathways, as well as transcription of specific defence genes.

Contrary to described elicitors of chewing arthropods, little is known about elicitors derived from sucking, phloem-feeding arthropods such as aphids and spider mites. Some of their orally-derived molecules might act as elicitors (e.g. oligogalacturonides) and it has been suggested that Ca^{2+} influx and membrane depolarisation is triggered as result⁴². Even though the early signalling mechanisms of these herbivores still needs to be clarified, it is well accepted that signalling pathways trigger primarily the salicylic acid (SA) response^{reviewed in 34,38}.

Other molecules involved in the stress response of herbivore attack are active nitrogen species (NO) and reactive oxygen species (ROS). Although the specific role of NO in herbivore defence signalling is not completely clear, it has been shown that its production and activity is closely related to the phosphorylation events, Ca^{2+} influx and activity of SIPK⁴³. ROS (O^{2-} , H_2O_2 etc.) burst in herbivore injured plants is thought to be related to Ca^{2+} ions, FACs and protein kinases activity³⁴.

In short, herbivore induced defence responses are activated through a complex, and not yet completely understood, cross talk of cell membrane depolarization, ion flux, mitogen activated protein kinases (MAPKs) activation, as well as induction and production of reactive oxygen species, nitric oxide and phytohormones (JA, SA, ethylene), result of which are numerous defence related responses, including emission of the HIPVs that can act as signalling molecules for neighbouring plants.

PATHOGEN INFECTION

Plants can also perceive volatile signals emitted from neighbours attacked by pathogens. Inoculation of

lima bean plants with avirulent strain of the biotrophic bacterial pathogen *Pseudomonas syringae* resulted in a defence response in neighbouring conspecific plants. Furthermore, the same response was observed when systemic acquired resistance (SAR) to the pathogen was artificially induced in emitting plants with application of benzothiadiazole⁴⁴. Shulaevet *al.* showed that tobacco plants *Nicotianatabacum* attacked by tobacco mosaic virus release VOCs capable of inducing defence response in their neighbours⁴⁵. Infection of maize leaves with four different pathogenic filamentous fungi species (*Fusarium spp.*) resulted in the release of several VOCs acting as plant-plant signalling molecules⁴⁶. As already seen for the herbivore attack, mechanical wounding of the emitting plant alone can be also insufficient to invoke a full response in receiving plants upon pathogen infection. Zeringue showed that cotton leaves (*Gossypiumhirsutum*) exposed to mechanically wounded and *Aspergillusjiauu*s infected leaves only fully respond to VOCs emitted from infested plants⁴⁷. This specific pathogen-induced release of signalling VOCs corresponds with mechanisms of plant immunity that are triggered upon pathogen attack. It is well established that plants are able to detect pathogen and microbe-associated molecular patterns (PAMPs and MAMPs) such as fungal chitin, bacterial lipopolysaccharides, peptidoglycans and flagellin with activation of the surface-localized pattern recognition receptors (PRRs) that can be either receptor-like kinases or receptor-like proteins ^{reviewed in 48}. Such activation results in a burst of ROS, Ca²⁺ influx, activation of Ca²⁺-dependent and mitogen-associated protein kinases, leading to the induction of SA (biotrophic pathogens) or JA and ethylene (necrotrophic pathogens) signalling pathways, induction of a defence response and potentially to a systematic acquired resistance. This primary immune response is called PAMP-triggered immunity (PTI) ^{reviewed in 49, 50}. Pathogens are able to suppress PTI with active molecules, called effectors, which can trigger the second line of the plant immune response (effector-triggered immunity, ETI). However, since ETI usually results in basal defence responses, such as localized programmed cell death⁵¹, it probably does not play an important role in inducing plant-plant signalling VOCs. On the other hand, it has been shown that mechanical wounding alone can trigger the release of interplant VOC signals²⁹ linked to pathogen

attacks. The same mechanism, as with herbivore attacks, is proposed here; plants are able to detect and respond to DAMPs. A question that arises from this is how are plants able to respond differently to artificial wounding i.e. herbivore or pathogen specific? To fully understand this specificity, more experiments should be performed, focusing on the unbiased monitoring of the whole range of responses to the mechanical wounding. Possible mechanisms behind induced specific responses could be based on pattern differences and even temporal properties of mechanical damage.

RESPONSE TO ABIOTIC STRESS FACTORS

There are only few studies published that indicate that plants can emit and respond to the volatiles, whose induction is based on abiotic stress factors. Being sessile organisms, plants are dependent on limited resources leading to constant competition with their neighbouring organisms. Allelopathy, phenomena when plants suppress growth or establishment of their neighbouring plants by emitting specific chemicals into the environment, is a known mechanism of plant competition⁵². There are few studies indicating that plants can also respond to non-allelopathic volatile signals from their neighbours, perceiving an increasing lack of space, resources and closeness of other plants and consequently responding with a change in growth. Ninkovicet *al.* showed that interplant VOCs mediated communication between two barley cultivars (Alva and Kara) affects biomass allocation in the individual plants⁵³. It has also been shown that tobacco plants can perceive and respond to the ethylene released by other plants when grown in high dense cultures^{54, 55}. Numerous abiotic stress factors, such as light, temperature, UV and radiation are known to influence the release of VOCs⁵⁶. Recently Yao *et al.* showed that UV irradiated *A. Thaliana* plants released volatiles that caused a response in neighbouring tobacco plants^{57,58}.

As we can see VOCs detected by neighbours are mainly produced as part of an immune response or as part of a response to different stress conditions, such as herbivore and pathogen attack, UV radiation and increasing lack of resources. It is not possible to show exactly what triggers the production of plant-plant signalling VOCs or what elements are involved in their induction and production pathways and how these differ from other regulatory pathways of induced defence. Furthermore, it is very likely (as

already mentioned in the introduction) that the primary function of such VOCs is not neighbour signalling, but rather more acknowledged defence responses (e.g. attraction of carnivorous insects) or within plant signalling. To better understand the

signalling and regulation of VOCs and their role in emitter plant defence, a better understanding of specific cues and signalling pathways is necessary.

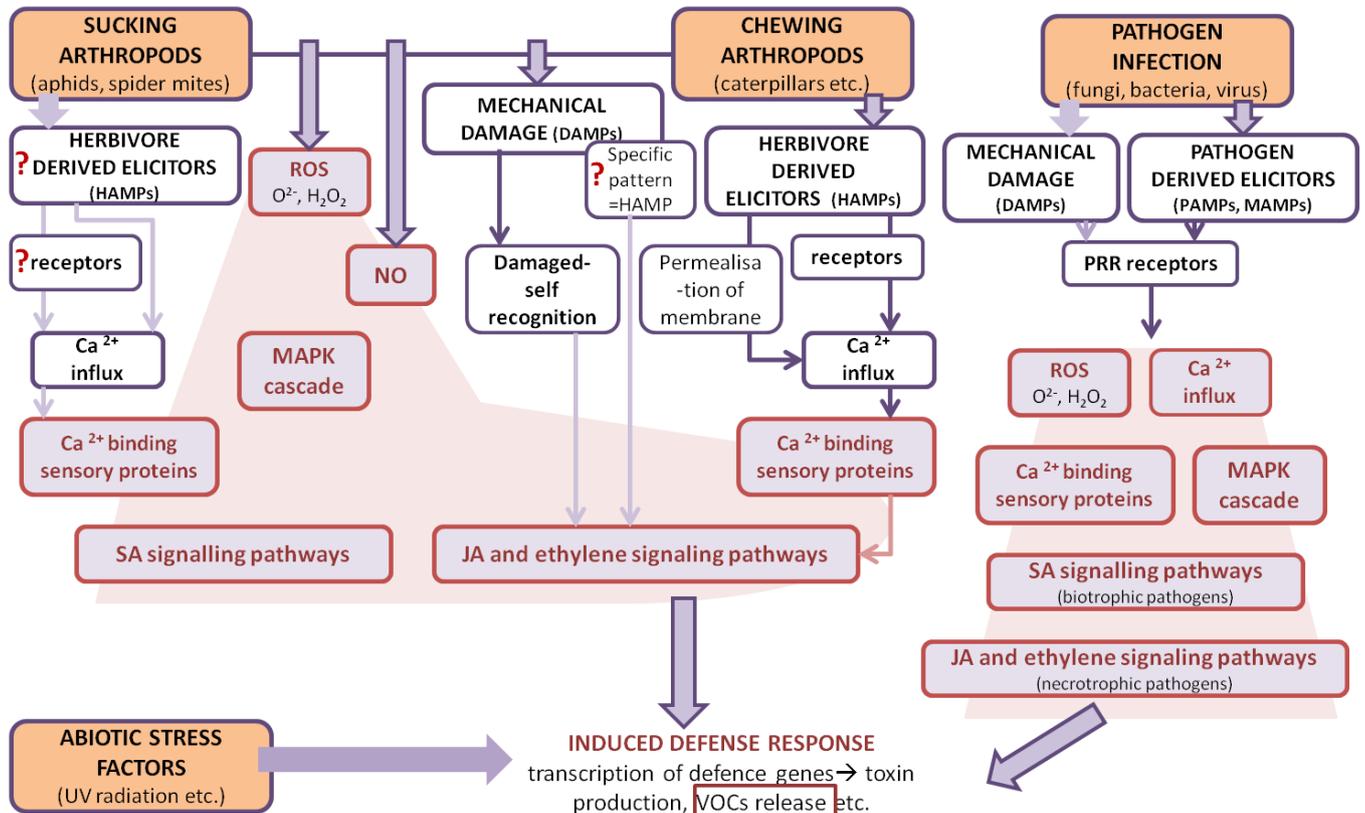


Fig. 2: Cues for release of VOCs responsible for interplant communication, their recognition and signalling pathways leading to the induction of VOCs. Herbivory, pathogen infection and abiotic stress factors have been shown to induce emission of VOCs that play a part in interplant communication. Faded arrows represent pathways that still need to be elucidated or confirmed. Shaded areas include elements involved in cross-talk.

Identity of signalling molecules

What is the identity of VOCs that were found to induce a response in the receiving plants? The most common VOCs involved in the interplant signalling are methyl salicylate, oxylipin metabolites methyl jasmonate and cis-jasmone as well as terpenoid and green leaf volatile compounds (**table 1**).

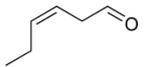
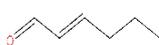
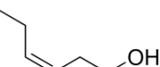
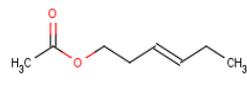
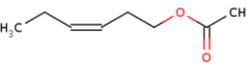
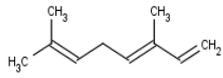
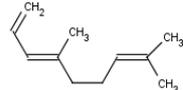
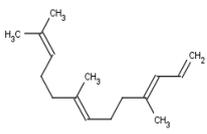
Terpenoids present the largest class of secondary metabolites in plants¹¹. They are synthesised via two independent alternative pathways from a five-carbon compound isopentenyl diphosphate (IDP) and its allylic isomer dimethylallyl diphosphate (DMAPP). The mevalonic acid (MVA) pathway takes place in the cytosol, while the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway occurs in the plastids^{reviewed in 11,37,61}. Several terpenes were

reported to be VOC molecules mediating the communication between several plant species; homoterpenes (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), monoterpenes (E)- β -ocimene and ocimene and sesquiterpenes (E)- β -farnesene (**Table 1**).

The second big group of interplant signalling VOCs are green leaf volatiles (GLVs) which are six-carbon compounds including the alcohols, aldehydes and their esters, formed via hydroperoxidelyase pathway of oxylipin metabolism^{reviewed in 62}. GLVs involved in the plant-plant signalling were shown to be released in several plant species in response to herbivore attacks or artificially induced mechanical wounding.

The most commonly released compounds are different forms of hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexen-1-yl acetate and (Z)-3-hex-3-enyl acetate (**Table 1**). Other VOCs that often play a part in plant-plant signalling are methyl salicylate (MeSA), phenolic compound of salicylic acid, and oxylipin metabolites methyl jasmonate (MeJA) and cis-jasmone. Less common are phytohormone ethylene, which was found to be emitted after the defoliation of black alder and from tobacco plants grown in high

densities^{8,54}, methanol, proposed to be a signal transmitter between tobacco plants upon mechanical wounding²⁹, methacrolein, emitted by *Artemisia tridentata* upon mechanical wounding³⁰, benzothiazole, an aromatic heterocyclic compound shown to act as a signal between populus trees²⁶ and nonanal, an alkyl aldehyde released from the lima bean plant in response to exposure to *P. syringae*⁴⁴.

	VOC	plant species	ecological context	ref.
green leaf volatiles	(Z)-3-hexenal 	<i>Chrysanthemum cinerariaefolium</i>	Mechanical wounding, laboratory	27, 28
	(Z)-hexenal	<i>Zea mays</i>	Mechanical wounding and herbivory (caterpillars), laboratory	25
	(E)-2-hexenal 	<i>Chrysanthemum cinerariaefolium</i> , <i>Artemisia tridentata</i> (as emitter), <i>Nicotiana attenuata</i> (as receiver), <i>Phaseolus lunatus</i>	Mechanical wounding and herbivory (spider mites), field and laboratory	27, 28, 30, 63
	(Z)-3-hexen-1-ol 	<i>Chrysanthemum cinerariaefolium</i> , <i>Zea mays</i> , <i>Phaseolus lunatus</i> , <i>Arabidopsis thaliana</i> ,	Mechanical wounding and herbivory (leafminers, caterpillars, spider mites), laboratory	7, 16, 25, 27, 28, 6
	(Z)-3-hexen-1-yl acetate 	<i>Zea mays</i> , <i>Chrysanthemum cinerariaefolium</i> , <i>Arabidopsis thaliana</i> , <i>Populus deltoides</i> × <i>nigra</i> , <i>Phaseolus lunatus</i>	Mechanical wounding and herbivory (caterpillars, spider mites), laboratory	7, 25, 27, 28, 63, 6
	(Z)-3-hex-3-enyl acetate 	<i>Phaseolus lunatus</i>	Herbivory (beetles), laboratory and field	65
	Several GLVs	<i>Triticum aestivum</i> , <i>Avena sativa</i> , <i>Hordeum vulgare</i>	Mechanical wounding and herbivory (<i>Oulema melanopus</i> , beetles), laboratory	17
terpenes	ocimene	<i>Phaseolus lunatus</i> (as emitter), <i>Arabidopsis thaliana</i> (as receiver)	Herbivory (leafminers), laboratory	16
	(E)-β-ocimene 	<i>Phaseolus lunatus</i> , <i>Alnus glutinosa</i>	Herbivory (spider mites), defoliation, laboratory and field	8, 13
	(3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) 	<i>Phaseolus lunatus</i> , <i>Arabidopsis thaliana</i> (as receiver), <i>Alnus glutinosa</i>	Herbivory (leafminers, spider mites), defoliation, laboratory and field	8, 13, 16
	(3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) 	<i>Phaseolus lunatus</i> , <i>Arabidopsis thaliana</i> (as receiver), <i>Alnus glutinosa</i>	Herbivory (leafminers, spider mites), defoliation, laboratory and field	8, 13, 16

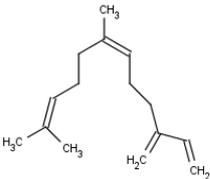
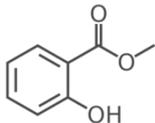
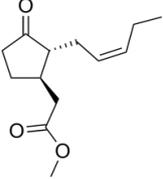
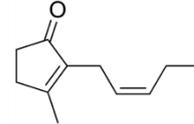
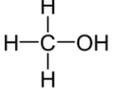
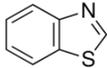
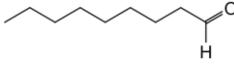
	(E)- β -farnesene 	<i>Chrysanthemum cinerariaefolium</i>	Mechanical wounding, laboratory	27,28
	Methyl salicylate (MeSA) 	<i>Nicotianatabacum</i> , <i>Arabidopsis thaliana</i> , <i>Phaseoluslunatus</i> , <i>Populussimonii</i> \times <i>Populuspyramidalis</i>	Mechanical wounding, UV radiation, exposure to pathogens (<i>Pseudomonas syringae</i> , tobacco mosaic virus), laboratory and field	26,44,45,58
	Methyl jasmonate (MeJA) 	<i>Artemisia tridentate</i> (as emitter), <i>Lycopersicomesculentum</i> (as receiver), <i>Nicotianaattenuata</i> (as receiver), <i>Arabidopsis thaliana</i> , <i>Populussimonii</i> \times <i>Populuspyramidalis</i>	Mechanical wounding, UV radiation, laboratory and field	23,26,30,58
	Cis-jasmone 	<i>Zea mays</i> , <i>Ribesnigrum</i> (as emitter), <i>Viciafaba</i>	Mechanical wounding and herbivory (caterpillars, aphids), laboratory	66 19,25 ,
other	Ethylene $H_2C = CH_2$	<i>Alnusglutinosa</i> , <i>Nicotianatabacum</i>	Defoliation, plants growing in high densities, field and laboratory	8,54
	Methanol 	<i>Nicotianabenthamiana</i>	Mechanical wounding, laboratory	29
	Methacrolein 	<i>Artemisia tridentata</i> (as emitter), <i>Nicotianaattenuata</i> (as receiver)	Mechanical wounding, laboratory and field	30
	Benzothiazole 	<i>Populussimonii</i> \times <i>Populuspyramidalis</i>	Mechanical wounding, laboratory	26
	Nonanal 	<i>Phaseoluslunatus</i>	Exposure to pathogen (<i>Pseudomonas syringae</i>), laboratory and field	44

Table 1: Identity of VOCs involved in interplant communication. VOCs are listed with plant species, known to emit and receive them, and ecological conditions, under which their release was induced.

Release of VOCs

When plants suffer herbivore or pathogen attacks, or are under the abiotic stress conditions, such as strong UV radiation, a mechanical wounding alone can already enable some volatile compounds to be released from the disrupted cellular compartments, where they were stored. Such VOCs are released from the damaged sites immediately. Such VOCs can be also emitted from the undamaged sites of plants with some time delay regarding the attack⁶⁷. VOCs can also be released as *de novo* synthesised compounds. This usually occurs as part of the systemic defence response. Such VOCs can also be emitted with some time delay from the undamaged parts of the plant.⁶⁷ They are released through leaf stomata and, in case of the lipophilic volatiles, such as terpenes, also through the membrane of epidermal tissues and from other structures, such as trichomes⁶⁸. Most of volatile compounds that take part in interplant communication are synthesised *de novo* or from the already stored precursors under control of the signalling pathways triggered by the previously described cues.

GLVs for example are emitted from the disrupted leaf and stem tissues very rapidly (within a few seconds to few minutes^{reviewed in 37}) after an attack. Such a rapid response is thought to result from the contact of substrate and enzymes that are under normal conditions stored in separate compartments yet encounter each other only after tissue damage. Regarding the substrate (free fatty acids) for the formation of GLVs, it has been suggested that they are formed upon tissue damage via hydrolysis of galactolipids. Decompartmentalization however is not always necessary for the formation of GLVs; GLVs were observed to be released also from the undamaged plant tissues as part of what is thought to be a systemic response of plants under herbivore attack^{reviewed in 37,62}. Terpenoids on the other hand are normally released with a several hour delay after attack, after induction of biosynthetic genes such as terpene synthase (TPS), resulting in complete *de novo* synthesis of the volatile compounds³⁷.

In order to convey a message to the neighbouring plants, released VOCs must be successfully transported to the receivers. Many of the initial experiments on interplant communication were performed under laboratory conditions, usually utilising concentrations of VOCs far above naturally occurring ones. As a result it was questioned whether aerial transport of tested compounds would

trigger the same response under field conditions, where not only the concentration would probably be lower, but also natural occurrences such as radiation, presence of air pollutants, wind and temperature changes could contribute to a lesser amount of volatile molecules reaching the receiving plant. Indeed, air pollution has been found to influence the transfer of signalling molecules; Blandeet *al.* showed that ozone concentrations commonly encountered in nature (80 ppb) significantly decreased the distance over which VOCs could be transmitted between lima bean plants. This was explained by ozone dependant degradation of HIPVs in the atmosphere⁶⁹. On the other hand, ozone was also shown to cause increased emission of VOCs⁷⁰. Some VOCs can be oxidised or otherwise processed in the atmosphere during transport. GLVs are oxidised by ozone, NO₃ and OH and are estimated to remain intact for only a few hours after release³⁷. Whether such modified VOCs have ecological function is unknown.

When considering VOCs another factor that has to be taken into account is the properties of volatile molecules themselves. Small molecules, such as ethylene, are easily diluted in the atmosphere as they diffuse very rapidly. Instead of long distance signalling over open space, ethylene, a singular signalling molecule, is more successful in signalling within enclosed spaces, such as under canopies⁵⁴. GLVs, terpenes, MeJA and MeSA on the other hand are heavier compounds with less volatility⁷¹ and consequently they do not diffuse in every direction but rather stay closer to the ground meaning that the release of lower concentrations of such volatiles could possibly still reach the receiving plants.

After initial laboratory oriented experiments, several studies were performed in the field, under natural conditions, conforming the transfer of signal for several VOCs and plant species (**table 1**). Karbanet *al.* observed how neighbours of clipped sagebrushes became herbivore resistant; they showed that a signal could be transferred as far as 60 cm from the emitting plant. Furthermore, they observed that most of the emitting sagebrushes had conspecific neighbours closer than that⁷². Heilet *al.* performed experiments on lima bean plants and observed transmission of airborne resistance up to 50 cm from the emitting plant, after which it dropped drastically, and at a distance of 100 cm no significant increase in resistance of plants was observed⁴. Transmission of

the signal for over 3 m from the emitting plant was observed between maize plants as well as between some other grasses, such as wheat, barley and oats⁴⁶.

Research in the last couple of years has shed new light on just how complex interplant signalling is. That plants can send specific messages and that receiving plants respond differently to different signals was mentioned already in the chapter about cues recognition. Plants can therefore send a message that will vary depending on the type or even species of the attacker. Furthermore, it has recently been shown that plants can respond differently depending on the (genetic) relatedness of the emitting plant⁹. This was demonstrated by a study performed using three annual plant species (*Lipinus nanus*, *Sanpis arvensis* and *Achyrrachaena mollis*) where defence responses of receiving plants growing next to damaged neighbours depended on genetic relatedness⁵.

How are plants able to emit and differentiate between all this different information utilising only a few so far described volatile compounds? It has become evident that signalling VOCs are usually released as specific blends of different compounds, often exhibiting specific concentrations of each of them reviewed in²⁸. *Chrysantenum cinerariaefolium* seedlings exposed to volatiles from damaged conspecific neighbours exhibited exactly the same defence response (i.e. induction of transcription of *13-LOX*, *DXS*, *CPP* and *AOD* genes as well as enhanced pyrethrin content) as seedlings exposed to the blend of volatiles matching concentrations and compounds of naturally released ones. However when even one of the five VOCs was removed from the blend, gene expression was drastically reduced. Furthermore, the blend of volatiles was only fully effective at specific concentrations; an increase or decrease of concentrations of VOCs resulted in reduced gene expression²⁷. Ruther *et al.* showed the other possible role of volatile blends by demonstrating that ethylene can act as a synergist to GLVs, inducing 2.5 fold increased defence response in maize plants when added to (Z)-3-hexenol⁷³. Different forms, i.e. epimers of the released volatiles, could also have role in different signal transmission. Cisepimer of MeJA, cis-MeJA, is biologically more reactive and therefore thermodynamically less stable, normally resulting in rapid epimerization to the more stable trans configuration. Approximately a 10 fold increase of

active epimer was observed after clipping a sagebrush plant in comparison to the control bushes⁶ and the trans to cisepimeric switch appeared to elicit defence responses in tobacco plants more effectively than either epimer alone⁷⁴. Such specific patterns of VOCs of different concentrations offer much wider variety of different possible signals and could be the answer to the coding and perception of numerous specific messages.

The concentration of volatile compounds seems to play an important role in plant-plant signalling. Perceived concentration of VOCs not only depends on the emitted concentration at a specific moment but also on accumulatively low signal concentrations over a long exposure time. Shiojiri showed that exposure to trace amounts (less than 140 pptV) of GLVs over 3 weeks induced defensive response in undamaged *A. thaliana* plant⁷. An experiment where lima bean plants were exposed to different concentrations of MeSA over 6 and 24 hours showed that an exposure to very low concentrations for longer exposure time resulted in a strong defence response, which was not observed when plants were exposed to the same concentration of VOC for a shorter period⁷⁵. These experiments indicate that a plant-plant signalling can involve an accumulation of the VOCs in the receiving plant. It is evident that the exposure time can be critical for a signal transfer, possibly enabling detection of signals with very low concentration over time. This could mean that the actual distance for a successful transfer of a signal could be greater than shown in experiments so far, provided VOCs are released for long enough time. Furthermore it opens a question whether some VOCs involved in the interplant signalling have been overlooked because of their low emission concentration. The bottom line is that such findings provide a good reference for the importance of the long-term experiments in the future studies.

RECEIVING AND RESPONDING TO THE MESSAGE

DIRECT RESPONSE TO THE VOLATILE SIGNALS

The idea of interplant communication was first triggered by the observations of the response actions carried out by the neighbours of attacked plants. Rhoades observed herbivore resistance of willow trees growing in close proximity of trees infested with tent caterpillars² and Baldwin and

Schultz reported increased concentrations of phenolic compounds in undamaged poplar ramets and sugar maple seedlings enclosed together with damaged conspecific plants¹. Numerous reports followed describing the induction of plant defence responses that were either exposed to volatiles emitting from neighbouring plants of the same or different species, or to the applied compounds corresponding the identity of VOCs, reported to be involved in interplant signalling.

A common response of receiving plants is acquired resistance, shown as a reduced damage by the attackers. Dolchet *al.* showed under field conditions that defoliation of alder trees resulted in a reduced amount of damage by alder leaf beetle and a reduced number of beetle eggs per leaf in the neighbouring trees²⁴. Air transfer experiments between clipped and undamaged sagebrush plants recently showed that unclipped plants treated with air from clipped ones exhibited reduced damage in response to naturally occurring herbivory⁷⁶. Volatiles emitted from whitefly infested tomato plants induced a bacterial resistance in neighbouring plants, showing cross-kingdom effect of the interplant signalling²⁰. Received VOCs were also shown to make plants more resistant to fungal disease⁷⁷, viral⁴⁵ and bacterial²⁹ infections. Several compounds, that are part of the induced direct defence response in receiving plants, were shown to accumulate or exhibit increased activity after exposure to interplant signals. Tomato plants exposed to MeJA, emitted from sagebrush plants, responded by increased production of the proteinase inhibitors²³. Black alders, shown to acquire resistance to herbivory when exposed to defoliated conspecific trees, also showed an increase in the activity of proteinase inhibitors and catalase when exposed to MeJA. Incubation with ethylene however resulted in production of phenolics, as well as proteinase inhibitors⁸. Wild tobacco neighbours of clipped sagebrushes were shown to have increased levels of polyphenol oxidase, a defensive oxidative enzyme, in case of a seasonal attack by cutworms and grasshoppers⁶. Hu observed that conspecific neighbours of *Populus simonii* *P. pyramidalis* cuttings showed increased levels of phenolic pyrochatechol, chlorogenic acid, gallic acid and p-hydroxyl benzoic acid²⁶.

Plants can respond to the received signals also by emitting volatile compounds themselves; as already mentioned, this can result in an indirect defence

response, such as the attraction of herbivore predators and parasitoid insects. It has been shown that cotton seedlings exhibit increased attraction to predatory mites when exposed to volatiles from seedlings, infested with herbivore mites⁷⁸. Increased attraction to predatory and parasitoid insects (i.e. ants and wasps) was also shown for lime bean plants exposed to the VOCs emitted from herbivore-damaged lima beans⁶⁵. Birkett observed that the application of cis-jasmone to intact lima bean plants induced production of several VOCs, including (E)- β -ocimene. As a consequence plants became more attracted to aphid parasitoids⁶⁶. Interestingly, newly emitted VOCs from the receiving plant can also attract pest insects; in a study performed on maize plants, exposed to volatiles from fungus infected conspecific neighbours, Piesik showed induced emission of several GLVs and terpenes and consequently increased attraction to insect pests *O. melanopus*⁴⁶.

Composition of VOCs, emitted from the receiving plants, is often similar to the volatiles that were shown to take part in the interplant signalling. This is not unusual, since it is very likely that the same volatiles that are emitted as part of the defence response, take part in several other modes of action (**Fig. 1**), such as within plant signalling and attraction of predatory insects. However one question that remains to be answered is whether these volatiles can also be perceived by the neighbouring plants and induce a response. Such a chain of action would mean that the message from the original emitter plant would be able to reach neighbouring plants over a much larger area.

Another question that we can ask concerns the mechanism by which VOCs are perceived. The similarity in the compositions of re-emitted and received VOCs from attacked plants suggest that response mechanisms of receiving plants are passive, simple re-emission of the received volatiles. Such passive adsorption and re-emission was observed in the case of birch trees (*Betula spp.*), which were shown to adsorb and re-emit arthropod-repelling terpenes, emitted by neighbouring *Rhododendron tomentosum*⁷⁹. Cholet *al.* showed similar results for conspecific plants; when lima bean plants were exposed to the volatiles of plants infested with spider mites, the emission of a blend of volatiles, similar to the one released from infested plants, was observed. Receiving plants were shown to emit volatile compounds even when their

production was inhibited with a protein-synthesis inhibitor. From this, the authors deduced that plants passively emitted volatiles that had previously been adsorbed. However leaves that were previously exposed to volatiles from infested leaves emitted larger amounts of volatiles and were also more attracted to predatory mites, which indicates that both the active and passive modes of action play a part in volatile release by receiving plants¹⁵.

It is well accepted that plants also actively respond to perceived signals; this is evident from the accumulation of defensive compounds in the receivers. Furthermore, several transcriptional analyses and monitoring of expression profiles identified numerous genes that show induced expression upon preception of volatile signals.

Activation of five defence genes, i.e. genes for the pathogen-related (PR) proteins PR-2 and PR-3, lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL) and farnesyl pyrophosphate synthetase (FPS), were detected in a study by Arimura *et al.*, who exposed lima beans to volatiles from conspecific leaves infested with spider mites¹³. The same genes were also expressed in lima beans when exposed to (E)-2-hexenal. Exposure to GLVs (Z)-3-hexenol and (Z)-3-hexenyl acetate resulted in different patterns of gene expression – (Z)-3-hexenol activated genes *PR-2*, *LOX*, *PAL* and *FPS* and (Z)-3-hexenyl acetate genes *LOX*, *PR-3* and *FPS*⁶³. Interestingly, only the transcript of *PR-2* gene was detected in receiving leaves exposed to VOCs emitted from mechanically damaged leaves¹³. This indicates that a different combinations of genes are triggered in the receiving plant depending on the signals and confirms that plants can not only emit cue specific signals, but also perceive them as different and act accordingly. Experiments using plants whose GLVs production was genetically silenced further showed that specific gene expression patterns can also be a consequence of the suppressive effect of some volatiles; comparison of gene transcriptional patterns of tobacco plants after exposure to GLVs-deficient and GLVs-complemented (resembling WT VOCs blend) volatile blend showed that numerous genes were under the negative regulation of GLVs⁸⁰. Among others, GLVs were also shown to induce expression of *PAL* gene, which plays part in MeSA synthesis, resulting in emission of MeSA volatile⁸¹, genes for chalcone synthase (*CHS*), caffeic acid-O-methyltransferase (*COMT*), diacylglycerol kinase 1 (*DGK1*), glutathione-S-transferase 1 (*GST1*) and

lipoxygenase 2 (*LOX2*)⁷⁷. An increase in *LOX* product (Z)-3-hexenyl acetate was shown to result in the conversion of exogenous GLV (Z)-3-hexenol, indicating that VOCs not only trigger response, but can also be further processed⁸¹. Furthermore, JA-dependent and independent pathways were suggested to play part in GLVs induced response⁷⁷. MeJA was shown to be responsible for upregulated transcription of *MPI* gene, i.e. gene expressing maize proteinase inhibitor (protein responsible for inhibition of digestive proteases of insects in maize plants), *IGL* gene (coding enzyme responsible for production of indole), *FPS* gene (coding enzyme responsible for synthesis of sesquiterpenes)⁸¹, *AOS* and *HPL*, coding enzymes in phytooxylipin pathway and *VSP1* gene that plays a part in ethylene and JA signalling pathways⁶⁰. By conversion into jasmonic acid and jasmonoyl isoleucine exogenous MeJA can also trigger signal transduction, leading to the activation of the defensive systems, e.g. VOCs emission in receiving plants⁸².

Allo-ocimene was shown to induce expression of genes for chalcone synthase (*CHS*), caffeic acid-O-methyltransferase (*COMT*), diacylglycerol kinase 1 (*DGK1*), glutathione-S-transferase 1 (*GST1*), lipoxygenase 2 (*LOX2*)⁷⁷, genes *AOS* and *HPL*, coding for enzymes playing part in phytooxylipin pathway, and *PR-3* and *VSP1* that play a part in ethylene and jasmonic acid signalling pathways⁶⁰.

Exposure to MeSA induced upregulated expression of *PR-1* gene, molecular marker for acquired resistance⁴⁵, as well as *PR-1* and *PR-2* genes, markers of salicylic acid signalling⁶⁰. MeSA itself can play a part in such signalling, as it has been shown that it can be converted back to salicylic acid⁴⁵.

Cis-jasmone triggered transcription profiles with upregulated genes for a cytochrome P450 and *CYP81D11*⁸³, and several genes that were shown to be under control of the transcription factors TGA2, TGA5 and TGA6, as well as regulatory protein SCARECROW-like 14 (*SCL14*), which could be a consequence of a specific regulatory pathway triggered by its perception⁸⁴.

Expression profiles of *A. thaliana* plants stimulated with methanol showed methanol dependent upregulation of 484 transcripts, which varied depending on the time during which samples were exposed to methanol, indicating activation of multiple signalling pathways at specific times. In general, most of the expression activity was detected in the areas of metabolism, cell communication, signal transduction processes and

defence genes⁸⁵. It has been recently shown that genes β -1,3-glucanase (*BG*), a previously unidentified gene (*MIG-21*), and non-cell-autonomous pathway protein (*NCAPP*) might be of specific importance, as they are especially affected²⁹.

Zhang *et al.* have recently published a full transcriptional analysis of *A. thaliana* in response to the leaf miner-induced volatiles from a lima bean plant as well as to the individual volatile compounds that were shown to be emitted from lima bean plants, presenting genome wide response to the interplant signalling. The study showed that transcriptional responses are positively correlated with treatment duration; only a few genes were enriched after a 24 hour treatment, all related to the defence responses. However, after 48 hours of treatment upregulation of numerous genes associated with both defense and metabolism pathways occurred. Furthermore, they have shown that ethylene and JA pathways are involved in detecting VOCs and that the ethylene pathway in the receiver plants is essential for communication¹⁶.

Gene transcription and expression analyses are able to provide some insight into which signalling pathways and which phytohormones are involved in the downstream detection of VOCs and in forming the defence response. Much less is however known about upstream signalling, perception of VOCs and the transduction mechanisms. Some volatiles, such as MeJA and MeSA, were shown to be converted to the JA or SA and therefore probably have the role of signalling molecules/phytohormones themselves. It is very likely that the activation of defence genes by VOCs can be also mediated via signalling processes such as protein phosphorylation/dephosphorylation, Ca^{2+} influx and burst of reactive oxygen species. There is some evidence that supports this assumption. Arimura *et al.* showed that the expression of monitored VOCs-dependent defence genes required Ca^{2+} influx and protein phosphorylation¹³. Furthermore Asai *et al.* demonstrated an increase of cytoplasmic free Ca^{2+} concentration in leaves of *A. thaliana* exposed to the volatiles. When plants were exposed to acyclic volatile compounds (β -ocimene, β -myrcene, DMNT), such a rise in concentration was shown to result in Ca^{2+} release from the intracellular compartments. In the case of (E)-2-hexenal however, Ca^{2+} influx was mediated via ROS production by natural oxidation⁸⁶, indicating that at least two different signal transduction processes are involved in forming VOC

induced responses. What is still unknown is the role of sensory proteins, most likely membrane-associated, able to detect VOCs and trigger further response. So far, the only known receptor able to detect volatile compounds in plants is a receptor for gaseous ethylene³⁸. Further research focusing on the early signalling of receiving plants is therefore needed for a better understanding of the molecular signal transduction.

PRIMING

Plants sometimes receive and perceive a signal, but develop a full defence response only after they are challenged by herbivores or pathogens. Signal therefore primes their defences for attacks yet to occur. Such response could be favourable for plant fitness, since preparing a defence response is quite energetically costly and its direct induction after the signal perception can mean energetic waste for plants if they are not challenged soon after. It looks like plants respond to the volatile signals in complex ways, often exhibiting a mix of priming and directly induced defence responses.

Engelberth *et al.* were the first to clearly demonstrate the priming phenomena for airborne signalling; they showed that corn seedlings, previously exposed to GLVs, produced more terpenoids and JA in comparison with non-exposed seedlings when mechanically damaged or induced with caterpillar regurgitant²⁵. Heil *et al.* showed similar results for the production of extrafloral nectar in lima bean plants; leaves that were previously exposed to volatiles from beetle-damaged conspecific shoots, showed a significantly higher production of extrafloral nectar when they were mechanically damaged in comparison to non-exposed ones¹⁰. Another example of priming as response to interplant airborne signalling was observed in a natural population of lima bean plants; when challenged with pathogen infection, plants previously exposed to the volatiles from neighbours with induced systemic acquired resistance showed significantly stronger expression of the pathogen-related protein 2 (PR-2) than non-exposed plants, and consequently exhibited better resistance⁴⁴. Priming was also observed in woody plants; hybrid poplars showed priming of JA, proteinase inhibitor and terpene volatiles synthesis⁶⁴.

Analyses of gene expression are consistent with before mentioned phenomena. Even when changes in the phenotype are not immediately observed,

transcription patterns of the defence related genes indicate that signals are perceived by the receiver plants. Microarrays enriched in herbivore-regulated genes of the native tobacco plants, neighbouring clipped sagebrushes, showed transcriptional responses, however presence of the defensive chemicals or proteins in tobacco plants was not detected. However when the same plants were challenged by application of caterpillars, accelerated production of trypsin protease inhibitors was observed³⁰. Similar non-direct activation of the genes was observed in maize plants, exposed to VOCs from caterpillar infested neighbours; 10 defence-related genes were identified, showing

stronger and earlier induction upon subsequent attack⁸⁷.

The mechanisms behind the airborne-induced priming are still mostly unknown⁸⁸. Jaskiewicz *et al.* showed that chromatin modification might play a role in the in-direct activation of genes. Treatment with an analogue of SA or pathogen infection induced chromatin modifications on promoters that are usually connected with activity of genes; however observed defence-related genes remained inactive. A strong correlation between histone modification patterns and gene priming was observed, indicating that histone memory could play a part in gene modifications relating to priming⁸⁹.

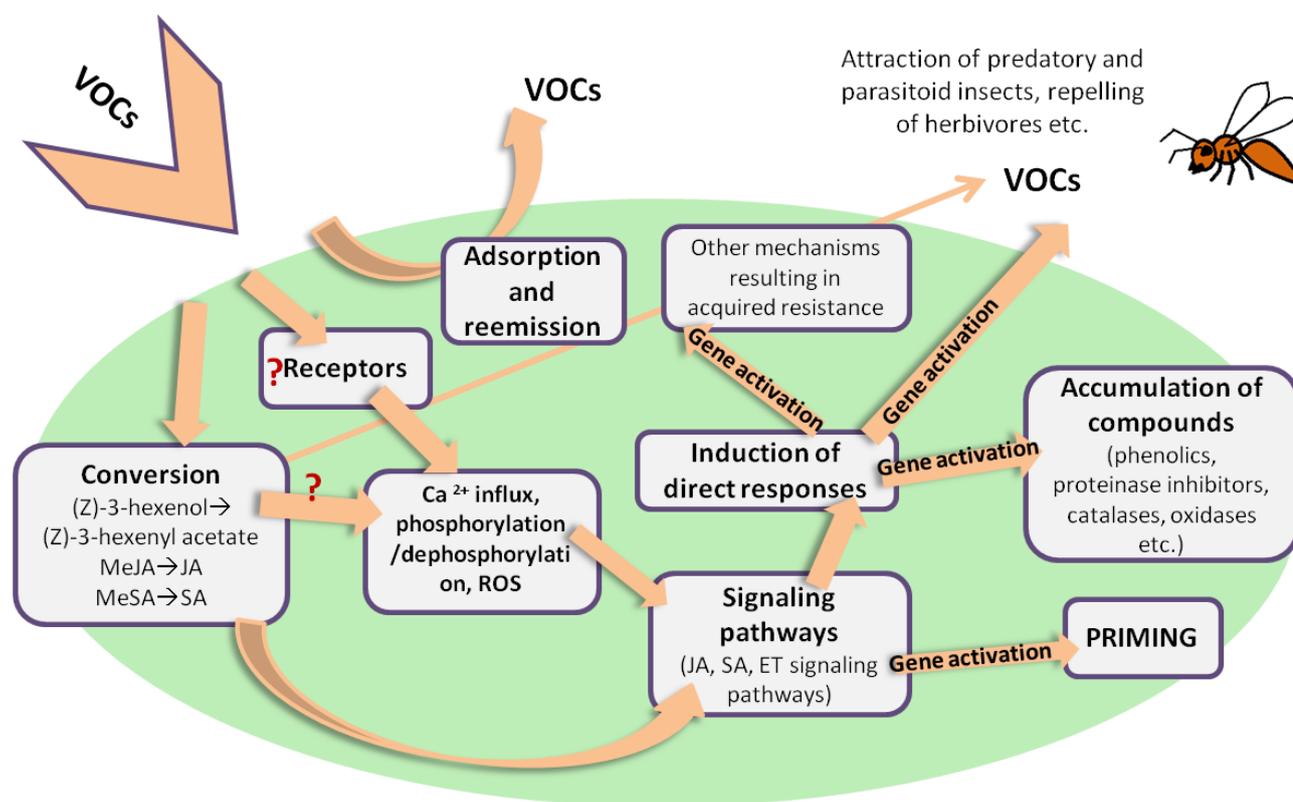


Fig. 3: Schematic overview of reported response actions of receiving plants to VOCs and indication of signalling mechanisms involved in VOCs perception further processing of signal. Received volatile signals can be either passively adsorbed and reemitted, or actively processed resulting in induction of defence response. This can be in form of direct induction of defence actions, such as emission of defence-related volatiles and accumulation of attacker inhibiting compounds, or in form of priming, phenomena when defence gene expression is fully activated only after plants are subsequently challenged. Green shaded area represents inside space of plant tissue.

Mechanisms of underground interplant communication

A significant part of the plant's structure lies underground. It is well known that this is where numerous interactions with other organisms, such as symbiotic microorganisms and fungi take place. It has also been shown lately that plants can actively shape and control such interactions⁹⁰. Despite that, there is still little known about the interplant interactions and possible communication below ground. Evidence for interplant communication has been so far found for the signalling through roots and common mycorrhizal networks; however most of the mechanisms behind them so far remain unknown.

Signalling through roots

It has been shown that roots respond to neighbouring roots in a specific and actively controlled manner ^{reviewed in 91}. Most studies on root based interaction between plants have focused on competition-based behaviour. Such interactions can depend on the species and the identity of the neighbours⁹². Different root-root behaviour was shown for different desert shrubs; roots of *Ambrosia dumosa* detect and avoid roots of conspecific plants, whereas *Larrea tridentata* roots exhibit inhibition of neighbouring *Larrea* and *Ambrosia* roots⁹³. Furthermore, there is an accumulating amount of the evidence that roots can recognise kin and relatedness of the neighbouring plants and respond accordingly.

Cakile edentula showed increased allocation to roots when groups of non-related plants were grown together. Such phenomena was not observed in case of the siblings⁹⁴, indicating that plants can discriminate kin in competitive interactions and that roots can very likely provide cues for such kin recognition. Root allocation is usually connected with the increase of below-ground competitive ability; therefore these results are consistent with kin selection. To better understand root recognition of plant neighbours at the molecular and biochemical level, Badriet *al.* recently analysed root secreted metabolites and proteins involved in early plant neighbour recognition, using *A. thaliana* co-cultured with different ecotype of *A. thaliana* or *Capsella rubella* neighbours. They observed a significant difference in secretion profiles of plants growing alone in comparison with plants growing next to a

neighbour; when grown alone, plants released a higher number of defence-related proteins, whereas plants growing with neighbours exhibited a decreased repertoire of these proteins and an increased secretion of specific defence-related proteins. Stress related proteins showed the reverse trend; both, the number and accumulative secretion of peroxidases decreased when plants were grown with neighbours. Furthermore, specific proteins were found to be only secreted when *A. dumosa* was neighbouring a specific neighbour⁹⁵. These findings indicate that plants are able to biochemically sense and distinguish similar or different neighbours. Ciszak *et al.* recently proposed that plant recognition abilities can result in the establishment of social behaviour; swarming behaviour was observed in alignment patterns of the roots of neighbouring maize plants. Root apices were observed to act as decision-making centres, establishing correlations in growth patterns⁹⁶. More data should however be obtained to confirm that root based interplant recognition can indeed lead to such social behaviour and if so, elucidate how this process is mediated.

Evidence exists that plants can also emit defence-related cues through roots that can be perceived and answered by neighbouring plants. Dicke *et al.* showed transfer of the herbivore-induced signal between lima bean plants. Lima bean plants infested with spider mites were placed in distilled water with their roots. Subsequently, roots of uninfested conspecific plants were placed in the same water, which resulted in their increased attraction to the predatory mites, indicating that above-ground herbivory can lead to the root changes and that chemical information can be transferred through soil⁹⁷. Even though this study is a good indication that root transfer of signals through soil is a likely possibility, it was performed under laboratory conditions. Plants were left in the water with their roots for seven days, which could have caused a physiological stress and consequently responses such as changed root permeability.⁹⁸

Similar observations were reported by Guerrieri *et al.*, who observed that healthy broad bean plants (*Vicia faba*) grown in the same pot as conspecific plants infested with the pea aphid exhibited increased attraction to the parasitoid *Aphidius ervi*. Such a phenomenon was not observed when root contact was prevented among plants. Furthermore, a defence response of intact plants was triggered when they were placed for 24 hours in hydroponic

solution in which infested conspecific plants were grown previously. Such response was not observed in the case of hydroponic solution previously used to grow intact plants, indicating that water soluble exudates from roots of infested plants are responsible for the signal transfer and triggering of the induced defence response in intact plants⁹⁹. In comparison to the research performed by Dicke *et al.*, time of exposure of uninfested plants to medium in which infested plants were grown was much shorter (24 hours versus 7 days^{97,99}), which could mean that different signalling pathways were taking part in signal transduction and recognition. Molecular mechanisms as well as identity of compounds, responsible for such communication, remain unknown. Furthermore, even though part of the mentioned experiments were executed using soil as medium, most of the discussed evidence for transport of defence cues remains based on the experiments using aqueous media and hydroponics. This might not accurately portray actual behaviour of plants; such artificial medium might affect root structure and plant response to environmental cues, since they present a different environment, lacking space and aggregates present in soil and not taking into account microbial activity or fluctuations in temperature, CO₂, ion concentration and water.

Herbivore attack is not the only topic of interplant root-root signalling. Recently Falik *et al.* showed that plants can also emit and perceive stress related signals. In their experimental set-up, individual split-root pea plants (*Pisium sativa*) were planted in two pots; separate roots were planted in an induction pot, which was subjected to osmotic stress and either in a pot with five or one target plant, sharing their rooting volume. In case of the one target plant, also split-rooted, one of the roots of target plant was further connected with a new target plant, enabling monitoring of the root signal transfer from induced plant through receiving plant to its neighbours. As targeting pea plants, bermuda grass *Cynodon dactylon*, hairy crabgrass *Digitaria sanguinalis* and buffalo grass *Stenotaphrum secundatum* were used. The response of the induced and neighbouring plants to the osmotic stress was monitored by recording the stomatal aperture kinetics. All of the target plants exhibited stomatal closure when they were neighboured with stressed plants, which indicates that unstressed plants are able to perceive and respond to the stress-induced cues emitted

from the neighbouring plants exposed to osmotic stress, and are also able to induce responses in additional unstressed plants. Plants might therefore be able to increase their readiness to future drought situations or osmotic stress. Furthermore, neighbouring plants were shown to acclimate to stress cues and re-open their stomata after a few hours, showing that such induced readiness is contemporary in absence of actual stress. This could be of relevance to fitness since open stomata plays an important role in gaseous exchange, which is an essential part of plant energy metabolism¹⁰⁰. Even though it is indeed very likely that the signal transfer was conducted through roots of plants, which were made to connect in a very specific manner, air space between plants was not limited. Stomatas of emitting induced plants were closed, which is also in favour of the root-root communication; however volatiles could be emitted and absorbed through plant tissue. Signal transport via air therefore cannot be completely ruled out in this case of the interplant signalling.

As we can see, there is strong evidence supporting the communication of plants through their roots. On the other hand, there is also data lacking on the mechanisms of molecular signalling pathways behind the signal transfer and on the identity of the signalling compounds.

Patterns of volatile response to herbivore attack are in some ways similar for both above and below ground. Below ground volatile emissions were shown to be able to protect plants by attracting entomopathogenic nematodes, much as above ground VOCs can do with attracting parasitoids and predatory insects. One such compound, sesquiterpene (E)- β -caryophyllene, was identified as an insect-induced below ground plant signal, emitted by maize roots in response to the feeding by beetle larvae. It exhibited high ability of the diffusion (up to 10 cm) in sand-containing moist soil, which, together with its chemical stability, seem to make it exceptionally suitable as a below ground signal¹⁰¹. Another below ground volatile, pregeijerene (1,5-dimethylcyclodeca-1,5,7-triene), was observed to be emitted from the citrus roots upon larval *Diaprepes abbreviatus* feeding and also shown to attract entomopathogenic nematodes¹⁰².

Given good diffusion rates and considering the examples from volatile above ground signalling, it may be that mentioned volatiles play a part in the interplant root to root communication; that they can

be perceived by roots of neighbouring plants and consequently induce their defence response. This is of course purely speculative; however it offers interesting opportunity for possible further studies. Furthermore, even if these specific volatiles play a role only in attracting parasitoidic nematodes, they still give clues to the type of molecule that may be worth looking into as possible candidates of root to root signalling.

Signalling through underground common mycorrhizal network (CMN)

Mycorrhizal fungi are commonly symbiotic, dependent on carbon uptake supplied by plants and providing nutrients, such as nitrogen and phosphorus to hosts¹⁰³. Numerous plants can be attached to the same network, creating opportunity for some plants to 'cheat'; contribute to less or none of the carbon to the network, however still uptake supplied nutrients. Interactions in form of the sanction mechanisms were reported to exist between plants and between plants and fungi in such cases¹⁰⁴. It has been recently shown that plants can also exhibit kin recognition in the mycorrhizal networks; mycorrhizal network size and root colonization were greater when sibling plants were connected in comparison to the non-related ones, resulting in the higher uptake of nutrients¹⁰⁵. Such kin selection could be an alternative to sanctions in prevention of the cheating, as investment of carbon in plants closely related could mean increase of the indirect fitness of plants. Common mycorrhizal networks (CMNs) also play part in the transfer of water and nutrients between plants. Such transfer of carbon between roots of ectomycorrhizal tree species *Betula papyrifera* and *Pseudotsuga menziesii* can be bidirectional and possibly regulated by a source-sink relationship¹⁰⁶.

It is increasingly acknowledged that CMNs can also enhance transport of allelopathic and signalling compounds between plants; a Network Enhanced Bioactive Zone model proposed by Bartoet *al.* suggests that CMNs act as superhighways, directly connecting plants below ground and therefore increasing the bioactive zones of the infochemicals. In comparison to the classical below ground signalling, such transfer of information could protect transported molecules from exposure to the soil environment and therefore prevent their fast degradation, sorption or formation of complexes

with metals, enabling slower decrease in their concentration over increased distance¹⁰⁷.

Several published studies support this model. So far studies have focused on the networks formed by arbuscularmycorrhizal fungi, i.e. fungi known to associate with more than 80% of the vascular plants and have low host specificity, commonly resulting in communities consisting of several species of plants¹⁰³. Experiments comparing accumulation of several hydrophilic and lipophilic allelopathic compounds after their diffusion either through the soil or through the CMNs showed that accumulation of compounds was greater when plants were growing in the soil with CMNs. Furthermore, CMNs significantly increased the distance through which compounds were able to commute; a gap of 1.5 cm was enough to prevent transfer of observed compounds through soil, whereas transfer through CMNs was observed in all tested distances between plants, up to a maximum of 12 cm¹⁰⁸. This indicates that CMNs might be especially important for transferring compounds of low water solubility in systems where roots of plants are far apart or are not in direct contact.

Interplant communication through CMNs has also been reported to be involved in the transfer of defence signals. Song *et al.* observed tomato plants (*Lycopersicon esculentum* Mill.) connected with a mycorrhizal network formed by the fungus *Glomus mosseae*. Direct root contact and above-ground air transfer was prevented between plants. When one of the plants, connected with a CMN, was infected with the pathogen *Alternaria solani*, neighbouring healthy plants exhibited increased disease resistance and activity of defense-related enzymes peroxidase, polyphenol oxidase, chitinase, β -1,3-glucanase, phenylalanine ammonia-lyase and lipoxygenase. Furthermore, receiving plants showed a significant increase in the expression of six defence genes: genes encoding tpathogen-related proteins *PR1*, *PR-2* and *PR-3*, phenylalanine ammonia-lyase (*PAL*), *LOX* and *AOC*. *PAL*, *LOX* and *AOC* play a part in SA and JA signalling pathways, indicating their role in the transduction of signals leading to an induced resistance in receiving plants¹⁰⁹. A study recently published by Babikova *et al.* used a very similar experimental approach to determine whether CMN can also affect transport of the signal, induced by herbivore infestation. Bean plants (*V. faba*) sharing a mycorrhizal connection with conspecific plants infested with pea aphids (*Acyrtosiphon pisum*), showed an increased attraction for the parasitoid

wasp (*A. ervi*); a phenomena that was absent when plants lacked CMN connections¹¹⁰.

Interplant communication with signal transport through CMN presents an attractive mechanism of plant-plant signalling, however more studies are required to confirm current findings. The identity of signalling compounds, mechanism of their release, perception as well as the molecular signalling pathways involved in the induction of defence responses, still need to be discovered. There is already some indication that the transport of signalling molecules could be cytoplasmic, following an active uptake by hyphae or passive across the fungal cell membranes. Alternatively, transport can be apoplastic, if the compounds diffused through the fungal cell wall, or surficial, if the compounds diffused through the layer of water on the surface of the hyphae. Mycorrhizal hyphae can create channels when intertwined together; such cords can be filled with water, which could also create a transport route for the signalling compounds¹⁰⁷. Whether any of these suggested mechanisms play a role in the signalling through CMNs is however yet to be determined.

Plants as whole organisms – integrated view on the interplant communication

As it has been discussed through this thesis, several types of interplant signal communications exist. However, most of experimental research focuses on only a single type of signal transport at a time and experiments have been designed in such a way that other types of signal transfer, except the tested one, are prevented. It has rarely been considered that other possible mechanisms of communication probably occur⁶. On the whole, it seems that experimental research on certain mechanisms of interplant communication has been overlooked. This appears to be especially true for the transport of signals through CMNs; in laboratory greenhouse experiments, sterile soil is very commonly used, which means that mycorrhizal fungi cannot develop. Furthermore, a development of CMNs between plants takes time, so even when natural soil is used, experiments performed are usually too short for connections to form.

Comparison of the published data shows that similar plants sometimes exhibited different types of interplant communication in different experiments.

Lima bean plants infested with spider mites are very commonly used as a model system for interplant communication. In this system, aerial transport of defence-inducing VOCs between neighbouring plants has been shown independently several times^{13–15,65}, always using only isolated above-ground growing plant parts in the experimental set-up. The exact same system of lima bean plants infested with spider mites was also used in aforementioned experiment performed by Dicke *et al.*, where signals were transmitted between plant roots in aqueous medium⁹⁷. Furthermore, attraction of predators, which was the response of neighbouring plants in the case of root to root signalling, was also described in three of the experiments with VOC mediated communication^{13,15,65}. Considering that cues for signalling and responses of receiving plants were the same in all cases, it is quite likely that signalling takes place above and belowground simultaneously.

Another plant, used in separate experiments describing different ways of interplant communication, is the broad bean (*V. faba*), reported to take part in root to root⁹⁹, as well as mycorrhizal mediated¹¹⁰, signalling in response to pea aphid infestations, which in both cases resulted in an increased attraction of the parasitoid wasp (*A. ervi*) to the receiving plant. In the root experiment, carried out by Guerrieri *et al.* group, the role of aerial transfer on interplant signalling was excluded by the experiment, where plants were placed in close proximity and the contact of the roots was prevented by placing plants in separate pots. Resulting responses of receiving plants were similar to the responses of the control plants⁹⁹. In an experiment by Babikova *et al.* on CMNs, aerial transfer was excluded by placing the receiver plants in polyethyleneterephthalate (PET) bags¹¹⁰. Interestingly, the experiment by Babikova *et al.* also excluded any significant influence of root to root signalling¹¹⁰, which is inconsistent with Guerrieri *et al.*'s root experiment, who used the same plant-herbivore system, resulting in the same response of receiving plants. Since Guerrieri *et al.* used sterile soil in the experiments⁹⁹, it is not very likely that root to root signalling was actually mediated by mycorrhizal connection. Considering all this, it is likely that both CMN and root to root interplant signalling can take place; however vary depending

on specific circumstances, such as the amount of time that has passed from induction of the signal. Separate experiments showed that tomato plants can communicate via mycorrhizal¹⁰⁹, as well as VOC mediated aerial transport²⁰. In these experiments, plants did not respond to the same cues or exhibit the same signal-induced response. Communication via VOC mediated aerial transport was a response to a greenhouse whitefly infestation²⁰, whereas CMN mediated signal transfer was triggered by an infection with a pathogen¹⁰⁹. Responses of the receiving plants were similar in both cases; receiving plants exhibited an increased pathogen resistance, in the case of mycorrhizal transfer to *Alterbaria solani*¹⁰⁹, whereas in the case of volatile mediated signalling to pathogen bacteria *P. syringae*²⁰. Signalling via both described ways simultaneously was prevented by blocking the air flow between plants in the case of the experiment on mycorrhizal signalling, and by growing plants in the separate pots during the experiment on VOCs aerial signalling. Given that in this system plants were responding to different cues in different experiments, it is possible that specific mechanisms of signal emission and transport are triggered in a way that is dependant up on the cue. However signals could also be simultaneously transported via both mediums, or in a time lag, possibly connected with the induction of the systemic defence response. Furthermore, induced pathogen resistance of receiving plants in both cases could mean triggering similar or same signalling pathways upon perception of the signal.

These examples open several new questions. First, can emitting plants respond to the same above or underground cues using different signalling mechanisms (above and below-ground signalling) that can be perceived by neighbours, and if so, how are these signals transferred between organs of emitting plants? Secondly, are receiving plants able to perceive different types of signalling through different mediums at the same time and if so, how do they integrate and process information?

In order to give a complete answer to the first question, experiments should be performed where different types of signal transport (above and below ground) are simultaneously observed. Experiments performed so far however have already given some insight. As already discussed, separate experiments with the same plant species indicate that same plants can use different types of interplant signalling simultaneously. Furthermore, that plants can emit

signals from different parts as the site where signal-inducing cue originated can be seen in most reported cases of underground communication^{97,99,109,110}. There is also evidence for the reverse situation; infection belowground resulted in VOC emission above ground⁴⁶. The importance of such responses for fitness of plants can be explained to some extent, since when attacked, either above or below ground, the whole plant is affected and is more susceptible from attacks in all directions. In already mentioned study by Dicke *et al.* such transfer can even be directly traced in the plant. Besides the already mentioned experiment, where the signal was shown to be transmitted through roots using aqueous medium, Dicke *et al.* performed two additional experiments on different parts of plants. In the first experiment, an infested leaf was placed in water with a petiole. Subsequently, an uninfested leaf was placed in the same water, resulting in an increased attraction to the predatory mites. In the second experiment, a lima bean plant, whose primary leaf was removed in a way that only the petiole remained, was attached to a vial filled with the distilled water. After seven days, water from the vials was collected and healthy leaves were placed in it, again resulting in an increased attraction to the predatory mites⁹⁷. These two experiments, combined with the already described root to root signalling one, could indicate that hydrophilic signals can be transferred through the whole plant and that such signals can induce a defence response in neighbouring plants at any stage of the transport from an infested leave to other leaves or roots.

A good indicator that plants can probably respond to the same attack with several types of interplant signalling, emitted from directly induced as well as remote plant organs, are provided by studies on chemical communication and integration of the defence signals above and below ground. These studies present possible mechanisms through which interplant signalling molecules could be transported through plants as well as proof that plants function as whole organisms, integrating and processing information from all parts simultaneously.

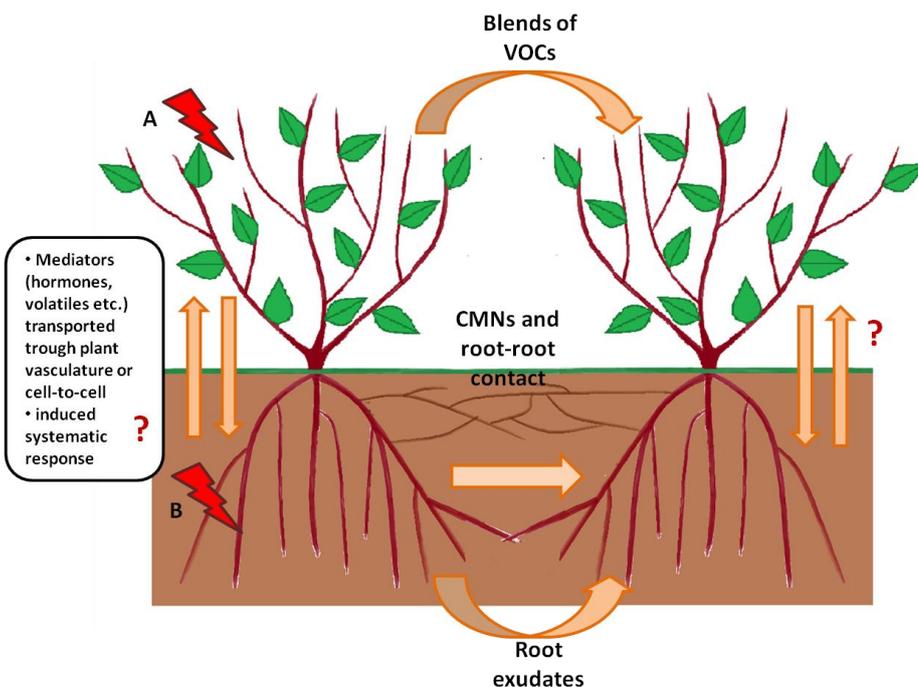
Below ground interaction of plants with plant growth-promoting rhizobacteria can prime plant resistance to an attack by herbivores and pathogens below as well as above ground. Such induced systemic response of plants is at least partially dependent on JA and ethylene signalling¹¹¹. Luthe *et*

al. showed an accumulation of defence gene transcripts in maize roots in response to caterpillar above ground feeding and proposed that such defence inducing cues from above ground sent a signal via the vascular system to the roots, which resulted in an accumulation of defence gene products below ground. Such a mechanism could act as a defence mechanism against belowground herbivory as well as a storage mechanism, enabling quick translocation of the active defence compounds¹¹². That roots are integrated in resistance and defence mechanisms triggered by above-ground herbivory was also shown by Kaplan *et al.* They showed that defence compounds can be synthesized in roots and translocated to the place of the attack above ground. Furthermore, plants also use roots as safe storage sites by translocating nutrients upon above ground attack¹¹³. An interesting review on the chemical communication between roots and shoots was written by van Dam and Bezemer, who showed an integrated view on above and belowground induced plant responses. They pointed out plants can be simultaneously

attacked in the leaves and roots, which could result in a cross-talk of defence responses to both attacks. They also underlined the importance of systemically induced responses for the responsiveness of the whole plants on a locally placed attacker and the importance of an integration of information from roots and shoots by plants for their fitness optimization on a multitrophic level¹¹⁴. Another relevant review, written by Erb *et al.*, focused on the interactions and integration of arthropod-induced above and below ground defence responses. They suggest that mediators, such as plant hormones, volatiles or nonhormonal metabolites could be transported through plant vasculature (xylem or phloem) and cell-to-cell signalling¹¹⁵.

Considering all this, we could deduce that emitting plants are likely to be able to send signals via different pathways and from different parts simultaneously. However, practical work has to be performed in order to test this and to determine mechanisms behind it.

Fig. 4: Schematic overview on the model of integrated interplant signalling.



Emitting plants are subject to either above (A), below (B), or simultaneously above and below ground stress factors, such as herbivory or pathogen infection. Consequently induced stress signals are transported and/or integrated through plant via mediators, resulting in induced systematic response. As part of this defence response, plants emit compounds that can be perceived by neighbouring plants. Such compounds can be emitted either above ground as blends of volatile molecules (VOCs), below ground as root exudates or via common mycotthizal networks (CMNs) or root-root contact. Receiving plants can probably respond to such signals in integrated manner.

Even less is known about the receiving plants ability to perceive different types of signalling through different mediums at the same time and how they would they integrate and process such information. So far, no study looked into this. Furthermore, since perception of singular interplant signals is not yet fully understood, nor the mechanisms behind induction of the defence response, it is even harder to provide an educated guess on how several signals, transported through different mediums, could be perceived and processed. There is an exciting opportunity for molecular ecologists and plant physiologists to fill this void.

Conclusions

A lot of the research has been done in the last few decades on interplant communication, showing that plants can emit and perceive signals above, as well as below ground and elucidating the identity of numerous signaling molecules and several mechanisms behind the emission and perception of them. However there is still a lack of information, especially on the molecular mechanisms of signalling pathways included in the signal emission, perception and processing. Furthermore, mechanisms of below ground plant to plant signalling remain mostly understood and still need to be elucidated.

So far plants have been rarely considered as integrated systems in the field of interplant signalling, which has mostly focused on their isolated parts. Ecological relevance of the interplant communication was sought by pressuring field based experimentation and explanations of evolutionary relevance, however in order for these studies to gain a complete ecological relevance, plants should be considered as whole organisms and integrated systems, in which above and below ground organs contribute to plant fitness together.

Therefore there is still a lot of work to be done in the fields of molecular ecology, genetics and plant physiology in order to better understand this interesting phenomenon

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